Biochemical and Biophysical Research Communications 427 (2012) 80–85

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Sulphoraphane, a naturally occurring isothiocyanate induces apoptosis in breast cancer cells by targeting heat shock proteins

Ruma Sarkar [a], Sutapa Mukherjee [a], Jaydip Biswas [b,1], Madhumita Roy [a,*]

[a] Department of Environmental Carcinogenesis & Toxicology, Chittaranjan National Cancer Institute, 37, SP Mukherjee Road, Kolkata 700 026, India
[b] Chittaranjan National Cancer Institute, 37, SP Mukherjee Road, Kolkata 700 026, India

ARTICLE INFO

*Article history:*
Received 23 August 2012
Available online 10 September 2012

*Keywords:*
HSP
HSF1
Sulphoraphane
Apoptosis
Breast cancer cells

ABSTRACT

Heat shock proteins (HSPs) are involved in protein folding, aggregation, transport and/or stabilization by acting as a molecular chaperone, leading to inhibition of apoptosis by both caspase dependent and/or independent pathways. HSPs are overexpressed in a wide range of human cancers and are implicated in tumor cell proliferation, differentiation, invasion and metastasis. HSPs particularly 27, 70, 90 and the transcription factor heat shock factor1 (HSF1) play key roles in the etiology of breast cancer and can be considered as potential therapeutic target. The present study was designed to investigate the role of sulphoraphane, a natural isothiocyanate on HSPs (27, 70, 90) and HSF1 in two different breast cancer cell lines MCF-7 and MDA-MB-231 cells expressing wild type and mutated p53 respectively, vis-à-vis in normal breast epithelial cell line MCF-12F. It was furthermore investigated whether modulation of HSPs and HSF1 could induce apoptosis in these cells by altering the expressions of p53, p21 and some apoptotic proteins like Bcl-2, Bax, Bid, Bad, Apaf-1 and AIF. Sulphoraphane was found to down-regulate the expressions of HSP70, 90 and HSF1, though the effect on HSP27 was not pronounced. Consequences of HSP inhibition was upregulation of p21 irrespective of p53 status. Bax, Bad, Apaf-1, AIF were upregulated followed by down-regulation of Bcl-2 and this effect was prominent in MCF-7 than in MDA-MB-231. However, very little change in the expression of Bid was observed. Alteration in Bcl-2 Bax ratio resulted in the release of cytochrome c from mitochondria and activation of caspases 3 and 9 which are in agreement with apoptotic index values. Sulphoraphane therefore can be regarded as a potent inducer of apoptosis due to HSP modulation in breast cancer cells.



## 1. Introduction

The eukaryotic stress response is highly conserved and involves the induction of heat shock proteins (HSPs). These families of proteins assist in the recovery from stress either by repairing damaged proteins or by assisting in their elimination [1]. HSPs restore protein homeostasis and promote survival of several client proteins regulating cell survival, proliferation and apoptosis [2]. Elevated expression levels of HSPs in malignant cells attribute a cytoprotective role by preventing apoptosis [3,4] and are closely associated with a poor prognosis [5]. Among different members HSPs (27, 70, 90) are mainly implicated in cancer progression [6]. HSP27 and HSP70 foster mammary tumorigenesis by inhibiting apoptosis and senescence [7], whereas HSP90 over-expression in breast cancer cells is a marker of poor prognosis [8].

HSP27 is associated with invasive potential of breast cancer [9], interacts with procaspase 3 and prevents its cleavage by caspase 9 [10]. It interferes with the extrinsic pathway of apoptosis by inhibiting Bid translocation [1,11] and directly binding to cytosolic cytochrome c and secluding it from apoptosis protease activating factor (Apaf-1) [1]. In murine fibrosarcoma cells, overexpression of HSP27 impeded cytoskeletal disruption and Bid intracellular redistribution that precede cytochrome c release [12].

HSP70 blocks caspase activation and suppresses mitochondrial damage and nuclear fragmentation [13] primarily by inhibiting Bax activation thereby plays a vital role in apoptosis [14]. HSP70 has been reported to bind directly to apoptosis protease activating factor-1 (Apaf-1), thereby preventing the recruitment of procaspase 9 to the apoptosome [15]. HSP70 interacts with procaspase-3 and 7, inhibiting caspase dependent apoptotic signaling [16]. Release of apoptogenic molecule AIF triggers caspase independent apoptosis [17] and HSP70 inhibits apoptosis by interfering with AIF [18]. HSP70 down-regulation sensitize tumor cells to apoptosis induction *in vitro* [19] and can reduce tumorigenicity *in vivo* [20].

HSP90, the most abundant protein in eukaryotic cells is required for the stabilities and functions of a number of signaling proteins [21]. HSP90 facilitates tumor progression by chaperoning the mutated or overexpressed oncogenes and signaling proteins involved in transformation and tumor progression [22]. HSP90α gene expression is elevated in breast cancer which is correlated with

* Corresponding author. Fax: +91 33 2475 7606.
*E-mail address:* mitacnci@yahoo.co.in (M. Roy).
[1] Director.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.09.006

decreased patient survival [8]. HSP90 can inhibit apoptosis by preventing the activation of caspases [10] and by directly binding to Apaf-1, preventing further recruitment of procaspase 9 [14]. Targeting HSPs therefore is an emerging concept in cancer therapy. Transcription of HSP gene requires an important transcription factor, heat shock factor1 or HSF1 which gets overexpressed and plays a vital role in the etiology of cancer [7,23].

Evidences show that sulphoraphane, a natural isothiocyante found in cruciferous vegetables have a wide range of chemopreventive as well as apoptosis inducing properties [24]. The ability of sulphoraphane in inducing apoptosis and cell cycle arrest is associated with regulation of many proteins including Bcl-2 family proteins, caspases, p21, and cyclin dependent kinases [25]. In human breast cancer cells, sulphoraphane treatment inhibits cell growth, activates apoptosis, inhibits HDAC activity and decreases the expression of key proteins involved in proliferation of cells [26]. This dietary isothiocyanate has been reported to inhibit breast cancer stem cells by downregulating the Wnt/β-catenin self-renewal pathway [24]. The present study evaluates the effect of sulphoraphane on HSPs (27, 70, 90) and HSF1 in two different breast cancer cell lines MCF-7 and MDA-MB-231 expressing wild type (wt) and mutated (mt) p53 respectively. MCF-12F, a non-tumorigenic breast epithelial cell line was used as control. The ultimate goal is to observe whether modulation of HSPs and HSF1 could induce apoptosis in these breast cancer cells by altering the expressions of certain proteins involved in apoptotic pathways.

## 2. Materials and methods

### 2.1. Chemicals

MEM, fetal bovine serum (FBS), acrylamide, *N,N′*-methylenebisacrylamide were obtained from Invitrogen BioServices India Pvt. Ltd., Bangalore, India. Ponceau S, dithiothreitol (DTT), phenylmethylsulfonylfluoride (PMSF), bovine serum albumin (BSA), ethylene glycol-*O*,-*O′*-bis,(2-aminoethyl) *N,N,N′,N′*-tetra acetic acid (EGTA), CHAPS, cytochalasin B, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), piperazine-*N,N′*-bis 2-ethanesulfonic acid (PIPES), proteinase K, propidium iodide (PI), RNase A, were purchased from Sigma Chemical Co, St. Louis, MO, USA; Sulphoraphane was purchased from M.P. Biomedicals, Germany, USA; goat anti-mouse IgG-alkaline phosphatase conjugate and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP-NBT) were purchased from Bangalore Genei, India; nitrocellulose membrane was purchased from Hybond ECL, Amersham Biosciences, UK; 17-AAG was procured from InvivoGen, San Diego, CA, USA. Tris, glycine, sodium dodecyl sulfate (SDS) were from Amresco, Ohio, USA; assay kits for caspase 3, caspase 8 and caspase 9 were purchased from Millipore, Billerica, MA and Caspase 9 inhibitor Z-LEHD-FMK was procured from BD Biosciences USA. The following antibodies were used for Western Blot analysis: HSP27, 70, 90, HSF1, caspase 3, caspase 8, caspase 9, cytochrome c, β-actin (Santa Cruz Biotechnology, California, USA); p53, p21, Bcl-2, Bax, Apaf-1, AIF, Bid and Bad (abcam, MA, USA). Other reagents used were of analytical grade and procured locally.

### 2.2. Maintenance of cell lines

Human breast adenocarcinoma cell line: MCF-7(ER+, EGFR−, weakly invasive, luminal epithelial like) and human metastatic breast adenocarcinoma: MDA-MB-231(ER−, PR−, HER2neu−, EGFR overexpressing, highly invasive, fibroblast like) were used in this study. They were routinely maintained in MEM and DMEM respectively supplemented with 10% heat inactivated fetal bovine serum (FBS) and antibiotics (gentamycin 40 μg, penicillin 100 units, streptomycin 10 μg/ml). Normal breast epithelial cell line MCF-12F was used as a control in this study and was maintained in a 1:1 mixture of DMEM and HAM's F12 medium with 0.4 mM $Ca^{2+}$, 20 ng/ml epidermal growth factor. Cells were allowed to grow at 37 °C in a humidified atmosphere of 5% $CO_2$/95% air.

### 2.3. Sulphoraphane treatment

Exponentially growing cells were treated with 1, 5, 10 and 20 μM sulphoraphane for 24 h.

### 2.4. Expression of HSPs (27, 70, 90), HSF1, p53, p21, Apaf-1, Bad, Bid, AIF, Bcl-2, Bax

Western Blot analysis was carried out to determine the expressions of HSPs, HSF1 and apoptosis related proteins p53, p21, Apaf-1, Bad, Bid, AIF, Bcl-2 and Bax using standard protocol [27] using specific antibodies.

### 2.5. Detection of cytochrome c release

Expression of cytochrome c in both mitochondrial and cytosolic fractions was determined by Western Blot analysis following the method of Roy et al. [27].

### 2.6. Determination of the activities of caspase 3, 8, 9

Aspartic acid specific cysteine proteases caspase 3, 8 and 9 activity was analyzed by a fluorimetric technique using fluorescent tagged peptides. The assay is based on detection of cleavage of synthetic substrates DEVD-AFC, IETD-AFC and LEHD-AFC (as supplied in kits) by caspase 3, 8 and 9 respectively to release free AFC. Free AFC emits a yellow green fluorescence at 480–520 nm (peak at 505 nm) upon excitation at 400 nm. Fluorescent signal was measured using a Spectrofluorimeter. Cell lysate prepared from cells exposed to sulphoraphane for 24 h was used to measure the activities following the protocol submitted by the supplier.

### 2.7. Measurement of apoptosis by PI staining

Morphological features of apoptotic cells as induced by sulphoraphane was examined by formation of apoptotic bodies and visualized with PI staining. Treated cells were harvested, washed with PBS and cell pellets were stained with PI (50 μg/ml). Stained cells were kept in the dark at room temperature for 10 min. Cells were spread on a slide and examined under a Nikon fluorescence microscope. Number of apoptotic and normal cells was counted in each slide and apoptotic index was calculated.

### 2.8. Statistical calculation

SPSS 10.0 software was used for statistical calculation using one way ANOVA followed by Dunetts *t*-test.

## 3. Results

### 3.1. Effect of sulphoraphane on expression levels of HSPs and HSF1

Western Blot analysis revealed that the expression of HSPs (27, 70 and 90) was high in MCF-7 and MDA-MB-231 cells compared to the normal breast epithelial cell line MCF-12F. Band intensities of the corresponding protein bands were calculated using Image Master Software and are depicted in Fig. 1A. Sulphoraphane (1, 5, 10 and 20 μM) down-regulated the expressions of HSP70, HSP90 dose

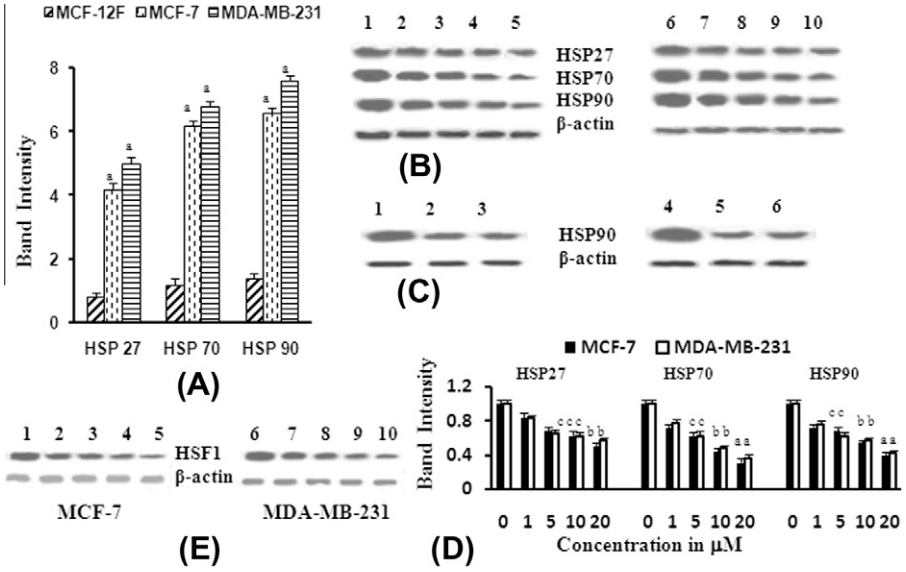


**Fig. 1.** Expressions of HSPs and HSF1 in MCF-7, MDA-MB-231 and MCF-12F cell lines and their modulation by sulphoraphane. (A) Band intensities of HSPs (27, 70, 90) expressed constitutively in MCF-12F (used as control) and in MCF-7, MDA-MB-231. Intensities calculated using Image Master Software is average of three independent experiments. Values represent mean ± SE and are significant [a]($p < 0.001$) with respect to control. (B) Modulation of HSPs in MCF-7 (lanes 1–5) and MDA-MB-231 (lanes 6–10) by sulphoraphane. Cells were treated with sulphoraphane at concentrations 0 μM (lanes 1, 6), 1 μM (lanes 2, 7), 5 μM (lanes 3, 8), 10 μM (lanes 4, 9) and 20 μM (lanes 5, 10) for 24 h. Western Blot analysis using anti-HSP27, anti-HSP70 and anti-HSP90 antibodies was performed. β-actin was used as control to ensure equal loading of protein. (C) HSP90 expression in MCF-7 (lanes 1–3) and MDA-MB-231 (lanes 4–6) cells after treatment with sulphoraphane and 17-AAG. Lanes 1, 4, lanes 2, 5 and lanes 3, 6 represented untreated cells, cells treated with 20 μM sulphoraphane and cells treated with 3 μM 17-AAG respectively. β-actin was used as loading control. (D) Band intensities of HSP27, 70, 90 in breast cancer cells as represented graphically. Values are average of three independent experiments and represent mean ± SE. They are significant [a]($p < 0.001$), [b]($p < 0.005$) and [c]($p < 0.01$) with respect to the untreated cells. (E) Constitutive expression of HSF1 (lane 1, 6) in MCF-7, MDA-MB-231 cells as determined by Western Blot analysis using anti-HSF1 antibody. Lanes (2–10) represented modulation of HSF1 expression after treatment with 1 μM (lanes 2, 6), 5 μM (lanes 3, 8), 10 μM (lanes 4, 9) and 20 μM (lanes 5, 10) of sulphoraphane.

dependently though very little effect on HSP27 was observed, Fig. 1B. Down-regulation of HSP90 by sulphoraphane (20 μM) was found to be comparable with that of 17-AAG (3 μM), a specific inhibitor of HSP90, Fig. 1C. Band intensities of different HSPs as modulated by sulphoraphane are represented graphically in Fig. 1D. HSF1 was found to be overexpressed in both MCF-7 and MDA-MB-231 cells as determined by Western Blot analysis using anti-HSF1 antibody (lanes 1 and 6 of Fig. 1E). Sulphoraphane down-regulated the expression of HSF1 in a concentration dependent manner (Fig. 1E).

### *3.2. Effect of sulphoraphane on the expressions of p53, p21 and apoptosis related proteins*

Expressions of p53 and p21, two important proteins that regulate G2-M transition of cell cycle were studied. Sulphoraphane increased the protein expressions of p53 and p21 in case of MCF-7 whereas in MDA-MB-231 (mutant p53), expression levels of p53 decreased significantly with a corresponding increase in the expression of p21 (Fig. 2A). This result indicated that up-regulation of p21 was independent of p53 expression. In order to investigate whether

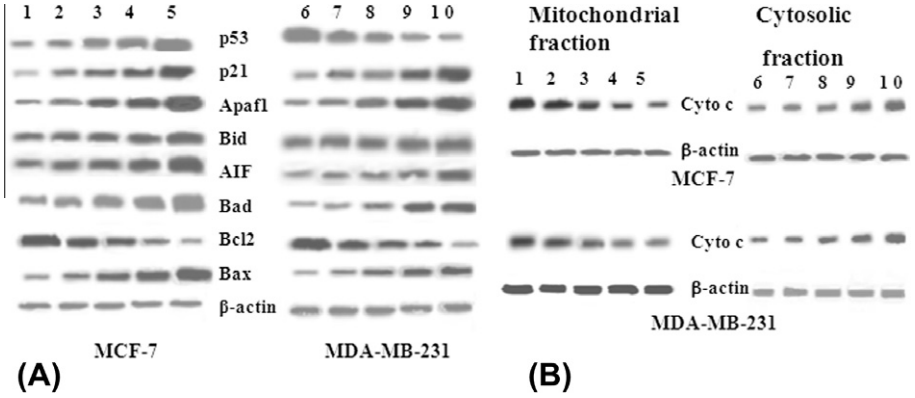


**Fig. 2.** Effect of sulphoraphane on certain apoptosis related proteins: p53, p21, Apaf1, Bid, AIF, Bad, Bcl-2 and Bax and cytochrome c release in breast cancer cells. (A) Lanes 1 and 6 represent constitutive expression of these proteins in MCF-7 and MDA-MB-231 cells. Cells were treated with sulphoraphane at concentrations 1 μM (lanes 2 and 7), 5 μM (lanes 3 and 8), 10 μM (lanes 4 and 9) and 20 μM (lanes 5 and 10) respectively for 24 h. Proteins were analyzed using specific antibodies and β-actin was used as loading control. (B) Expression of cytochrome c in MCF-7 and in MDA-MB-231cells. Lanes 1 and 6 represented expression of cytochrome c in mitochondrial and cytosolic fractions in untreated cells. Lanes 2–5 represented the expression of cytochrome c in the mitochondrial fraction and lanes 7–10 represented the similar expression in the cytosolic fraction after treatment with 1 μM (lanes 2 and 7), 5 μM (lanes 3 and 8), 10 μM (lanes 4 and 9) and 20 μM (lanes 5 and 10) sulphoraphane for 24 h. Cells were harvested, fractionated into mitochondria and cytosol and proteins from each fraction was analyzed for cytochrome c by Western Blot using anti-cytochrome c antibody. β-Actin was used as control to ensure equal loading of protein.

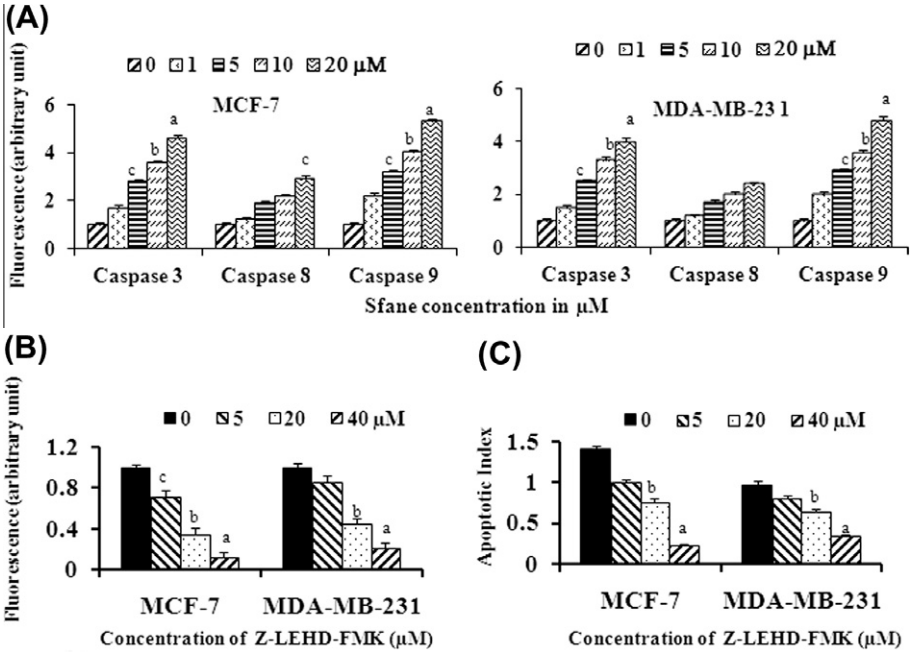


**Fig. 3.** Induction of caspase activity by sulphoraphane in breast cancer cells. (A) The activities of caspase 3, caspase 8 and caspase 9 in MCF-7 and MDAMB 231 cells subsequent to treatment with sulphoraphane for 24 h. The protease activity was determined fluorimetrically using fluorescent-labeled synthetic substrates of caspases 3 and 8 and 9. Values obtained are average of three independent experiments ± SD. They are significant [a]($p < 0.001$), [b]($p < 0.005$) and [c]($p < 0.01$) with respect to the untreated cells. (B) Effect of caspase 9 inhibitor on sulphoraphane induced caspase 9 activation in MCF-7 and MDA-MB-231. Cells were pretreated with different concentrations (5, 20 and 40 μM) of synthetic caspase 9 inhibitor (Z-LEHD-FMK) for 4 h followed by treatment with sulphoraphane (20 μM) for an additional 24 h. Values are average of three independent experiments ± S.D and are significant [a]($p < 0.005$) and [b]($p < 0.01$) with respect to control. (C). Apoptotic index as calculated in MCF-7 and MDA-MB-231 cells treated with caspase 9 inhibitor Z-LEHD-FMK (0, 5, 20 and 40 μM) for 4 h followed by treatment with 20 μM sulphoraphane for an additional 24 h. Values represent mean ± SD ($n = 100$) of three independent experiments. They are significant [a]($p < 0.005$) and [b]($p < 0.01$) with respect to control.

inhibition of HSPs by sulphoraphane could affect apoptosis, expressions of some of the important apoptotic proteins like Apaf-1, Bid, AIF, Bad, Bcl-2, Bax were observed. Expression of Apaf-1 was very poor in breast cancer cells and its expression increased with concentrations of sulphoraphane. However, expression of Bid was not much influenced by sulphoraphane (Fig. 2A). Western Blot analysis was performed to ascertain whether inhibition of HSP70 could induce the release of AIF. Results (Fig. 2A) showed that expression of AIF in cells exposed to sulphoraphane was high particularly at the highest concentration (20 μM). The effect was more pronounced in MCF-7 than in MDA-MB-231. Expressions of pro (Bax and Bad) and anti apoptotic protein (Bcl-2) were analyzed and results showed that sulphoraphane increased the expression of Bax and Bad, with a concomitant decrease in Bcl-2 expression (Fig. 2A). Cytochrome c release from mitochondria to cytosol was apparent in sulphoraphane treated cells as revealed by Western Blot analysis (Fig. 2B) in both the breast cancer cells. However, no such change was observed in MCF-12F (data not shown).

### 3.3. Activation of caspases by sulphoraphane

Fluorimetric assay of caspase 3, 8 and 9 in cells treated with sulphoraphane revealed that the activity of caspase 3, 8 and 9 increased with increasing concentrations of sulphoraphane. The degree of induction of caspase 8 was less than the other two caspases (Fig. 3A), indicating that induction of apoptosis was predominantly through intrinsic pathway. To establish the role of caspase 9, cells were treated with Z-LEHD-FMK (a potent cell-permeable caspase 9 inhibitor) for 4 h followed by treatment with sulphoraphane for an additional 24 h. Results revealed that this inhibitor blocked caspase 9 activation in a dose dependant manner, and sulphoraphane failed to induce caspase 9 activity in presence of Z-LEHD-FMK (Fig. 3B). To confirm the assumption that apoptosis induction is due to caspase 9 activation, apoptotic index was studied with Z-LEHD-FMK treated cells. The ratio of apoptotic and non-apoptotic cells (apoptotic index) showed that the apoptotic index values were reduced significantly with increasing concentrations

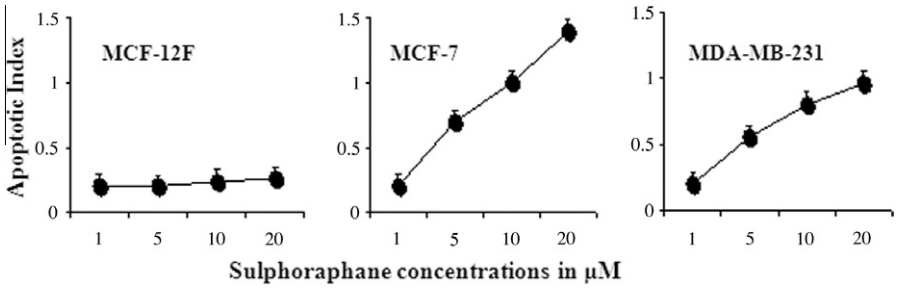


**Fig. 4.** Induction of apoptosis in sulphoraphane treated cells following PI staining. Apoptotic indices for MCF-12F, MCF-7 and MDA-MB 231 cells treated with different concentrations of sulphoraphane for 24 h. Result represented an average of three independent experiments. The ratio of the apoptotic to the non-apoptotic cells was designated as apoptotic index. Values represent mean ± SD ($n = 100$).

of inhibitors, confirming the involvement of caspase 9 in sulphoraphane induced apoptosis in breast cancer cells (Fig. 3C).

### 3.4. Sulphoraphane induced apoptosis as revealed from apoptotic index

Morphological features of apoptosis were observed with sulphoraphane treated cells and results (Fig. 4) depicted that the apoptotic index values increased dose dependently and induction of apoptosis was more in MCF-7. However, in MCF-12 cells, no increase in apoptotic index was observed.

## 4. Discussion

The anticancer efficacy of sulphoraphane has been evaluated in various cancers due to its beneficial pharmacological effects including antioxidant, anti-inflammatory, antitumor as well as apoptosis induction [28]. Sulphoraphane has been reported to inhibit cellular proliferation and apoptosis induction in different tumor cells both *in vitro* and *in vivo* [24]. High expression of HSP is essential for cancer survival; therefore inhibition of HSPs is an important strategy of anticancer therapy. HSF1 acts as a major enhancer of tumorigenesis. The present study demonstrated that sulphoraphane induced apoptosis in breast cancer cells by modulating HSPs. Expressions of HSP27, 70, 90 and HSF1 was efficiently down-regulated by sulphoraphane, though its effect was differential. Both HSP70 and 90 are involved in stabilization of mutant p53 in cancer [29]. Sulphoraphane was shown to up-regulate wild type p53 expression in MCF-7 cells whereas it down-regulated the expression of dysfunctional p53 in MDA-MB-231 cells. However, up-regulation of p21 in both the cell lines was observed irrespective of p53 status. Apaf-1 is involved in apoptosome formation, leading to apoptosis. Sulphoraphane efficiently induced Apaf-1 expression in breast cancer cells. Both HSP70 and 90 are known to suppress apoptosis by directly associating with Apaf-1 and blocking the assembly of procaspase 9 [14,15]. Expression of AIF, a flavoprotein gets up-regulated in presence of sulphoraphane. AIF is a novel caspase-independent death effecter protein which gets released from the mitochondria upon apoptotic stimuli. Previous studies have indicated that HSP70 interacts with AIF, thereby prevents its release [18]. Bid, a pro-apoptotic member of the Bcl-2 family proteins is known to trigger cell death via mitochondrial membrane permeabilization [30]. HSP27 has been shown to interfere with intracellular redistribution of Bid protein and inhibits the release of cytochrome c [12]. In the present study Bad was found to be induced by sulphoraphane. In response to apoptotic stimuli Bad rapidly gets dephosphorylated and migrates to the mitochondria where it induces cell death [31]. The anti-apoptotic action of HSP27 and 90 has been reported to be mediated by Akt/PKB, a protein that generates a survival signal in response to growth factor stimulation by phosphorylating Bad [14]. Increase in the expression of Bax protein with concomitant down-regulation of Bcl-2 protein by sulphoraphane as observed in the present study also supports the fact that inhibition of HSPs is associated with induction of apoptosis. Previous studies have shown that HSP27 and 70 inhibit release of cytochrome c by interacting with Bax protein [32]. HSP90, on the other hand stabilize Bcl-2 protein [33]. Present study indicates that inhibition of HSPs and HSF-1 probably led to up and down-regulation of pro-apoptotic and anti-apoptotic proteins resulting into the release of cytochrome c. This was associated with activation of caspases, particularly caspases 3 and 9. This was further supported by using specific inhibitor of caspase 9 where sulphoraphane failed to induce the activity of cysteine protease. Apoptotic index values further supported this notion.

The present study provides new insight into the mechanism of sulphoraphane induced apoptosis by targeting HSPs in breast cancer cells.

## Acknowledgments

The authors are grateful to Council of Scientific and Industrial Research (CSIR), Government of India, New Delhi, India for financial support and Director, Chittaranjan National Cancer Institute, Kolkata, India for providing infrastructural facilities.

## References

[1] A.A. Khalil, N.F. Kapaby, S.F. Deraz, C. Smith, Heat shock proteins in oncology: diagnostic biomarkers or therapeutic targets?, Biochim Biophys. Acta 86 (2011) 89–104.
[2] D. Lanneau, M. Brunet, E. Frisan, E. Solary, M. Fontenay, C. Garrido, Heat shock proteins: essential proteins for apoptosis regulation, J. Cell. Mol. Med. 2 (2008) 743–761.
[3] H.M. Beere, 'The stress of dying': the role of heat shock proteins in the regulation of apoptosis, J. Cell Sci. 117 (2004) 2641–2651.
[4] D.R. Ciocca, S.K. Calderwood, Heat shock proteins in cancer: diagnostic, prognostic, predictive and treatment implications, Cell Stress Chaperones 10 (2005) 86–103.
[5] S.K. Calderwood, M.A. Khaleque, D.B. Sawyer, D.R. Ciocca, Heat shock proteins in cancer: chaperones of tumorigenesis, Trends Biochem. Sci. 31 (2006) 164–172.
[6] D. Lanneau, A. de Thonel, S. Maurel, C. Didelot, C. Garrido, Apoptosis versus cell differentiation: role of heat shock proteins HSP90, HSP70 and HSP27, Prion 1 (2007) 53–60.
[7] S.K. Calderwood, Heat shock proteins in breast cancer progression – a suitable case for treatment, Int. J. Hyperthermia 26 (2010) 68–685.
[8] E. Pick, Y. Kluger, J.M. Giltnane, High HSP90 expression is associated with decreased survival in breast cancer, Cancer Res. 67 (2007) 2932–2937.
[9] P. Casado, P. Zuazua-Villar, E. del Valle, C. Martínez-Campa, P.S. Lazo, S. Ramos, Vincristine regulates the phosphorylation of the antiapoptotic protein HSP27 in breast cancer cells, Cancer Lett. 247 (2007) 3265–32659.
[10] P. Pandey, R. Farber, A. Nakazawa, S. Kumar, A. Bharti, C. Nalin, R. Weichselbaum, D. Kufe, S. Kharbanda, HSP27 functions as negative regulator of cytochrome c-dependent activation of procaspase-3, Oncogene 19 (2000) 1975–1981.
[11] S.J. Charette, J.N. Lavoie, H. Lambert, J. Landry, Inhibition of Daxx-mediated apoptosis by heat shock protein 27, Mol. Cell Biol. 20 (2000) 7602–7612.
[12] C. Paul, F. Manero, S. Gonin, C. Kretz-Remy, S. Virot, A.P. Arrigo, HSP27 as a negative regulator of cytochrome c release, Mol. Cell Biol. 22 (2002) 816–834.
[13] C.Y. Li, J.S. Lee, Y.G. Ko, J.I. Kim, J.S. Seo, Heat shock protein 70 inhibits apoptosis downstream of cytochrome c release and upstream of caspase 3 activation, J. Biol. Chem. 275 (2000) 25665–25671.
[14] E. Schmitt, M. Gehrmann, M. Brunet, G. Multhoff, C. Garrido, Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy, J. Leukoc. Biol. 81 (2007) 15–27.
[15] H.M. Beere, B.B. Wolf, K. Cain, D.D. Mosser, A. Mahboubi, T. Kuwana, P. Tailor, R.I. Morimoto, G.M. Cohen, D.R. Green, Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome, Nat. Cell Biol. 2 (2000) 469–475.
[16] E.Y. Komarova, E.A. Afanasyeva, M.M. Bulatova, M.E. Cheetham, B.A. Margulis, I.V. Guzhova, Downstream caspases are novel targets for the antiapoptotic activity of the molecular chaperone HSP70, Cell Stress Chaperones 9 (2004) 265–275.
[17] E. Daugas, S.A. Susin, N. Zamzami, K.F. Ferri, T. Irinopoulou, N. Larochette, M.C. Prevost, B. Leber, D. Andrews, J. Penninger, G. Kroemer, Mitochondrio-nuclear translocation of AIF in apoptosis and necrosis, FASEB J. 14 (2000) 729–739.
[18] L. Ravagnan, S. Gurbuxani, S.A. Susin, C. Maisse, E. Daugas, N. Zamzami, T. Mak, M. Jattela, J.M. Penninger, C. Garrido, G. Kroemer, Heat-shock protein 70 antagonizes apoptosis-inducing factor, Nat. Cell Biol. 3 (2001) 839–843.
[19] A.J. Massey, D.S. Williamson, H. Browne, J.B. Murray, P. Dokurno, T. Shaw, A.T. Macias, Z. Daniels, S. Geoffroy, M. Dopson, P. Lavan, N. Matassova, G.L. Francis, C.J. Graham, R. Parsons, Y. Wang, A. Padfield, M. Comer, M.J. Drysdale, M. Wood, A novel, small molecule inhibitor of HSC70/HSP70 potentiates HSP90 inhibitor induced apoptosis in HCT116 colon carcinoma cells, Cancer Chemother. Pharmacol. 66 (2010) 535–545.
[20] A.-L. Rerole, J. Gobbo, A. De Thonel, E. Schmitt, J.P.P. de Barros, A. Hamman, D. Lanneau, E. Fourmaux, O. Deminovi, O. Micheau, L. Lagrost, P. Colas, G. Kroemer, C. Garrido, Peptides and aptamers targeting HSP70: a novel approach for anticancer chemotherapy, Cancer Res. 71 (2011) 484–495.
[21] S.J. Myung, J.-H. Yoon, B.H. Kim, J.-H. Lee, E.U. Jung, H.-S. Lee, Heat shock protein 90 inhibitor induces apoptosis and attenuates activation of hepatic stellate cells, J. Pharmacol. Exp. Ther. 330 (2009) 276–282.
[22] S.B. Cullinan, L. Whitesell, Heat shock protein 90: a unique chemotherapeutic target, Semin. Oncol. 33 (2006) 457–465.
[23] A. de Thonel, V. Mezger, C. Garrido, Implication of heat shock factors in tumorigenesis: therapeutical potential, Cancers 3 (2011) 1158–1181.

[24] Y. Li, T. Zhang, H. Korkaya, S. Liu, H.-F. Lee, B. Newman, Y. Yu, S.G. Clouthier, S.J. Schwartz, M.S. Wicha, D. Sun, Sulphoraphane, a dietary component of broccoli/broccoli sprouts, inhibits breast cancer stem cells, Clin. Cancer Res. 16 (2010) 2580–2590.
[25] Y. Zhang, L. Tang, Discovery and development of sulphoraphane as a cancer chemopreventive phytochemical, Acta Pharmacol. Sin. 28 (2007) 1343–1354.
[26] A. Pledgie-Tracy, M.D. Sobolewski, N.E. Davidson, Sulphoraphane induces cell type-specific apoptosis in human breast cancer cell lines, Mol. Cancer Ther. 6 (2007) 1013–1021.
[27] M. Roy, S. Mukherjee, R. Sarkar, J. Biswas, Curcumin sensitizes the effect of chemotherapeutic drugs via modulation of protein kinase C, Telomerase, NFκB and Histone Deacetylase in breast cancer, Ther. Deliv. 2 (2011) 1275–1293.
[28] M.-J. Chen, W.-Y. Tang, C.-W. Hsu, Y.-T. Tsai, J.-F. Wu, C.-W. Lin, Y.-M. Cheng, Y.-C. Hsu, Apoptosis induction in primary human colorectal cancer cell lines and retarded tumor growth in SCID mice by sulphoraphane, Evid. Based Complement Alternat. Med. 2012 (2012) 415231.
[29] P. Muller, R. Hrstka, D. Coomber, D.P. Lane, B. Vojtesek, Chaperone-dependent stabilization and degradation of p53 mutants, Oncogene 27 (2008) 3371–3383.
[30] N. Zamzami, C.E. Hamel, C. Maisse, C. Brenner, C. MunÄ oz-Pinedo, A-S. Belzacq, P. Costantini, H. Vieira, M. Loeer, G. Molle, G. Kroemer, Bid acts on the permeability transition pore complex to induce apoptosis, Oncogene 19 (2000) 6342–6350.
[31] F.A. Sinicrope, R.L. Rego, N.R. Foster, S.N. Thibodeau, S.R. Alberts, H.E. Windschitl, D.J. Sargent, Proapoptotic Bad and Bid protein expression predict survival in stages II and III colon cancers, Clin. Cancer Res. 14 (2008) 4128–4133.
[32] A. Havasi, Z. Li, Z. Wang, J.L. Martin, V. Botla, K. Ruchalski, J.H. Schwartz, S.C. Borkan, HSP27 inhibits Bax activation and apoptosis via a phosphatidylinositol 3-kinase-dependent mechanism, J. Biol. Chem. 283 (2008) 12305–12313.
[33] C. Wong, S. Chen, Heat shock protein 90 inhibitors: new mode of therapy to overcome endocrine resistance, Cancer Res. 69 (2009) 8670–8677.